UNIVERSITY OF PENNSYLVANIA

CYTOPLASMIC INCLUSIONS IN THE GLANDULAR EPITHELIUM OF THE SCUTELLUM OF TRITICUM SATIVUM AND SECALE CEREALE

A DISSERTATION

IN BOTANY

PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL IN

PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR

THE DEGREE OF DOCTOR OF PHILOSOPHY

JOHN A. O'BRIEN, JR.

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CYTOPLASMIC INCLUSIONS IN THE GLANDULAR EPITHELIUM OF THE SCUTELLUM OF TRITICUM SATIVUM AND SECALE CEREALE [1]

John A. O'Brien, Jr.

ALTHOUGH THERE have been many attempts in recent years to determine the structural basis of secretion in animal gland cells, there has been very little investigation of the gland cells of plants in which modern cytological techniques were employed. While there is some agreement among biologists as to the rôles of the Golgi apparatus and mitochondria in relation to the secretory activity of animal cells, the very existence of the Golgi material in plants is questioned, and there is as yet no agreement as to the possible homologue of the apparatus in the vegetable cell. The mitochondria in plants have also been assigned a number of different functions, so it becomes highly desirable that plant gland cells be investigated further. Previous research on these cells has been confined principally to the proteolytic-enzyme-secreting cells of the carnivorous plants and to the epithelial gland cells of the scutellum of the grains. The present work is limited to an investigation of the morphological changes occurring within the cells of the latter, particularly with respect to the mitochondria. It is to be recalled that the cells in question have a two-fold function, absorption and secretion, a distinction that the majority of previous workers failed to make.

Plant physiologists early advanced evidence that the scutellar epithelial cells were gland cells, although the endosperm from which the embryo had been removed could itself digest its stored starch. The glandular nature of these cells has been generally accepted and is supported by more recent investigations of the physiology of germinating grains (Grünfeld, 1926; Schander, 1934).

Cytoplasmic granules have been reported in the epithelial cells of the scutellum of barley by Brown and Morris (1890) and of corn by Torrey (1902) and Reed (1904). These investigators suggested, directly or indirectly, a similarity between the granules and the zymogen granules of animal gland cells. Guilliermond (1908) observed an increase in basophilic granules in the epithelial cells of rye, barley, maize and wheat during germination and suggested a possible correlation between their presence and secretory activity. He later (1933) maintained, however, that the secretion of diastase produced no appreciable cytological alterations in the epithelial cells, and that the basophilic granules were merely mitochondria altered by fixation. Horning and Petrie (1927) observed an increase in the number of mitochondria by division in the epithelial cells of maize, wheat and barley, both in fixed cells and in living cells stained with Janus green. On the theory that mitochondria are the site of enzyme synthesis, they made the interesting suggestion that these bodies with the associated enzymes actually migrate from the epithelial cells into the endosperm, where, in contact with the starch grains, they disappear as the enzymes are liberated. As the identification of mitochondria in living plant cells by vital staining is very uncertain and as Janus green, together with most basic dyes, stains certain types of plant vacuoles (Bailey and Zirkle, 1931), the nature of these migratory inclusions seems somewhat uncertain. Newcomer (1938) demonstrated the presence of polymorphic and filamentous mitochondria in the epithelial cells of germinating maize but could find no evidence of their migration from the cell.

MATERIALS AND METHODS.—The plants investigated were *Triticum sativum* and *Secale cereale*. The grains were germinated in the dark on moist filter paper in Petri dishes. Since no attempt was made to keep the germinating grains at a constant temperature, the several stages of germination were identified by relative lengths of the radicle and the shoot rather than by the length of time which had elapsed since the germination was initiated. Observations were confined to fixed sections.

Zirkle (1929) suggested the use of the term mitochondria to include all those small cytoplasmic inclusions preserved by bichromates on the basic side of pH 4.2–5.2 and destroyed by more acid fixatives and by mixtures of bichromates and acetates. The pH range has since been narrowed to 4.8–5.2. This definition, used frequently in the literature, includes plastid primordia. In the epithelial cells under investigation, however, those inclusions which were observed to give rise to plastids are sufficiently differentiated in size and shape from the non-plastid forming mitochondria to warrant their designation as plastid primordia. In the present investigation, therefore, the term mitochondria is limited to those bodies included in the above definition which were not observed to give rise to plastids. The term plastid is applied only to those inclusions containing starch. The bichromate fixative used was Zirkle's (1934) modification of Erliki's potassium bichromate-copper sulfate mixture, composed of 1.25 gm. $K_2Cr_2O_7$; 1.25 gm. $(NH_4)_2Cr_2O_7$; 1.00 gm. $CuSO_4$ and 200.00 gm. H_2O. To this mixture enough pyridine was added to make a 0.50 per cent solution. The resulting precipitate was allowed to settle before the fixative was used.

[1] Received for publication January 13, 1942.

The author wishes to express his sincere gratitude to Professor Conway Zirkle for his suggestions and helpful criticisms during the course of this investigation and to Professors J. R. Schramm and William Seifriz for criticism of the manuscript. Part of this investigation was made during the tenure of a Harrison Fellowship in botany at the University of Pennsylvania.

For observations on the vacuole and its contents as well as the mitochondria, the above mixture, to which was added, in place of pyridine, 40 per cent formaldehyde to make 4 per cent, was employed. For the same purpose Bensley's (1910) formalin-bichromate-sublimate mixture was used in the following proportions: 2.50 gm. $K_2Cr_2O_7$; 5.00 gm. $HgCl_2$; 90.00 gm. H_2O. To this solution was added 40 per cent formaldehyde to make 4 per cent. After fixation in these last two solutions, the mitochondria did not retain the stain in the degree that they did after treatment in modified Erliki's-pyridine mixture, and in many sections they could not be observed. For

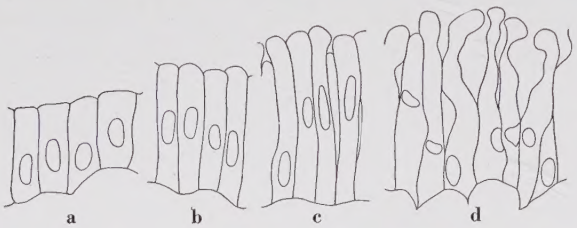

Fig. 1. Longitudinal sections of epithelial cells in grains of *Triticum* at successive stages of germination showing changes in cell size and shape; a, cells in resting grain; b, cells in germinating grain, the shoot of which has lengthened to 30 mm.; c, cells in grain with shoot 75 mm. in length; d, cells at end of germination; shoot 150 mm. in length. Irregular shape and lateral separation are evident. All figures ×300.

preserving the vacuole and its contents alone, a chromic sulfate-formalin mixture was used in the following proportions: 5 gm. $Cr_2(SO_4)_3 \cdot 15\ H_2O$; 0.5 gm. CuO; 90.00 gm. H_2O. To this solution was added 40 per cent formaldehyde to make 4 per cent (Zirkle, 1928).

Hardened endosperm made sectioning difficult. The young plants with scutella intact were, therefore, removed from the grain before fixation. The specimens were placed in the fixatives for forty-eight hours, after which they were washed in water and dehydrated in butyl alcohol (Zirkle, 1930) and embedded in paraffin. Sections were usually cut 5 micra in thickness. Heidenhain's iron-alum hematoxylin was the only stain employed.

Illustrations were made with the aid of a camera lucida.

Cytoplasm of the epithelial cells and its inclusions.—As germination progresses, there is a gradual increase in the length of the epithelial cells of the scutellum accompanied by a separation of the lateral walls as was first described by Brown and Morris (1890) and substantiated by later workers (fig. 1). Greater surface exposure is thereby provided for secretion and absorption, the specialized functions of these cells. In both rye and wheat, a greater lengthening occurs in the cells in the anterior or shoot region of the scutellum. Since the embryo is situated in the posterior portion of the grain, that part of the epithelial layer opposite the radicle is regarded as the posterior region and that part opposite the plumule, the anterior region. In the later stages, when the endosperm is approaching depletion, the epithelial cells appear as elongated, irregular projections, many of which are almost entirely separated from all adjoining cells (fig. 1d). The distal end assumes a variable form, sometimes developing what Horning and Petrie (1927) described as hypha-like protuberances (fig. 1d). It is to be noted that the lengthening of cells could be correlated only broadly with any particular stage in the germination process. Thus, occasionally, the epithelial cells of one grain are longer and display more advanced morphological changes than the epithelial cells of another grain with a shoot of equal length.

Resting stage.—*Aleurone grains.*—In the resting stage of rye and wheat grains, the short, closely placed epithelial cells contain a very dense and usually non-vacuolate cytoplasm. All the fixatives used disclose the presence of numerous, small, clearly delineated bodies dispersed in the cytoplasm (fig. 2). These structures stain lightly and irregularly and probably represent a fixation image of the aleurone grains which Guilliermond (1908) demonstrated to be present in the cells. The fixatives used also disclose in the scutellar parenchyma of wheat and rye larger aleurone grains similar to the grains in the epithelial layer. Occasionally a few epithelial cells in a section contain grains stained intensely. More generally, however, the stain is retained only slightly so that in many cases, particularly after fixation in Bensley's mixture or chromic sulfate-formalin fixative, an internal differentiation is observed in which a dark periphery inclosing non-stained areas can be discerned. Guilliermond identified the darkly staining portion as the amorphous protein of the aleurone grain while the non-staining areas represent globoids inclosed within the grain.

Mitochondria.—The presence of mitochondria in the epithelial cells cannot be demonstrated with any certainty during the resting stage. The dense cytoplasm and the dispersion of numerous small aleurone grains make delineation of any such bodies difficult. Horning and Petrie (1927) and Newcomer (1938) reported the presence of granular mitochondria in the resting stage of maize, the former after using an osmo-chromic fixative, the latter after fixing in modified Erliki's solution. That most of the inclusions represented in figure 2 are not mitochondria can be demonstrated readily by fixing the cells in a solution which will not preserve mitochondria. For this purpose a mixture of 0.5 per cent chromic acid and 1 per cent acetic acid was used. In the cells of a number of specimens thus fixed the inclusions were preserved as deeply staining discrete bodies, thus further substantiating the observation of Guilliermond (1908) that the bodies are aleurone grains. Undoubtedly they are not mitochondria. Since none of the fixatives used preserved the mitochondria, yet destroyed the aleurone grains, it cannot be stated definitely that mitochondria are present during the resting stage. That they are present, however, but masked by the aleurone grains can be assumed, since their occurrence can be demonstrated in the wheat

grain before the resting stage while the grain is still developing on the wheat plant.[2]

During the initial hours of germination, while the grain is swelling, little change is noted in the epithelial cells. Numerous aleurone grains are still preserved intact. Mitochondria are still not discernible, although Guilliermond reported the presence of basophilic granules from the first hours of germination.

STAGE I.—*Vacuoles.*—After germination continues for twenty-four hours, at which time the radicle has usually just broken through the outer coverings of the grain, marked changes occur in the epithelial cells. In numerous cells, small round or irregularly shaped vacuoles dispersed throughout the cytoplasm are evident, many of which enclose one or several small darkly staining granules (fig. 3). Guilliermond demonstrated the formation of vacuoles through the swelling and dissolution of the aleurone grains. The dark granules in the vacuoles at this stage are probably the fixation images of the precipitated remains of the aleurone grain protein. In some specimens at this stage a number of cells still retain intact numerous aleurone grains.

Plastids and mitochondria.—The most striking difference brought about after twenty-four hours germination is the presence of numerous inclusions which are preserved by the mitochondrial fixatives. At this and the following stage, two types of inclusion are discernible. Representing the first type are many large, usually rod-shaped bodies dispersed throughout the cytoplasm (fig. 4). Often these inclusions are knobbed structures. Rather than rod-shaped, they sometimes appear rounded off and shortened (fig. 4). Frequently they contain round, optically clear vesicles which are often present at the end of the rod (fig. 4). At a later stage (IV), to be referred to, the vesicles in these inclusions can be identified as starch grains by means of a polarizing microscope. Thus these bodies are plastid primordia or, if starch is present, plastids. The presence of such structures should be expected, for Guilliermond (1908) and Toole (1924) reported the presence of starch in the epithelial cells during germination.

Representing the second type of inclusion present at this stage is a profusion of minute granular mitochondria (fig. 4). These bodies which are dispersed throughout the cytoplasm were never observed to develop into plastids.

Neither of these two types of inclusion shows any orientation which would suggest its passage through the end wall of the cell as reported for mitochondria in the epithelial cells by Horning and Petrie (1927). Instead they are present in equal numbers in all parts of the cytoplasm.

All of the above mentioned inclusions retain the stain deeply after fixation in modified Erliki's-pyridine mixture. They stain only lightly when Bensley's solution is used; moreover, the plastid primordia and plastids are much contracted after fixation in this mixture (fig. 16).

STAGE II.—*Vacuoles.*—As germination continues and the shoots reach a length of 4 to 5 mm., the number of small vacuoles increases. In the majority of cells larger vacuoles appear, particularly in the basal part of the cell, due probably to the swelling and coalescing of smaller ones, as the number of vacuoles per cell decreases (fig. 5, 6). In the distal part of the cells the vacuoles, without undergoing any great increase in size, assume a tortuous, canalicular form, many of which are orientated parallel to the longitudinal axis of the cell (fig. 5, 6). In general, then, the cytoplasm appears more dense in the distal part of the cell than in the basal part. As the cytoplasm in these cells has been observed in rapid cyclosis, perhaps the shape of the vacuoles indicates only the condition of cytoplasmic movement at the moment of fixation and not some secretory function of the vacuole. The precipitated remains of the aleurone grains are still evident in some cells (fig. 5, 6).

As previously indicated in stage I, there is no marked change in the mitochondrial or plastid complex at this stage (fig. 4).

STAGE III.—*Vacuoles.*—As the shoots increase from 5 to 15 mm. in length, the cells become more vacuolate, large vacuoles forming in both the basal and distal parts of the cell as a result of further increase in size and coalescence. Often a single large vacuole, surrounded by a thin layer of peripheral cytoplasm, is formed in the extreme basal region. This vacuole, in the fixed cell, is usually empty (fig. 7) or contains, at most, a few strands of precipitated vacuolar material. While many specimens at this stage contain numerous cells with the empty basal vacuole, its presence is more characteristic of the next and later stages. In the enlarged vacuoles in the distal region there is contained a vacuolar material which is preserved usually as a lightly staining, homogeneous mass (fig. 7). This substance usually completely fills the vacuole so that often it cannot be delineated readily from the surrounding cytoplasm, especially after fixation in modified Erliki's-formalin or Bensley's mixture. While all the fixatives used preserve the vacuolar contents, in many specimens, particularly after fixation in chromium sulfate-formalin mixture, the vacuolar material is precipitated in irregular clumps (fig. 8), although an occasional cell in the same section will have vacuoles containing the homogeneous precipitate (fig. 9). In some cells, the basal vacuoles also contain the precipitated material (fig. 8). In vacuoles of many cells, usually one or occasionally two or three darkly staining, spherical granules are also preserved. They either occur free in the vacuole (fig. 12, 13) or are embedded in the non-staining vacuolar material, if both are present in the same vacuole (fig. 23, 25). Although preserved by all the fixatives here used, they are particularly evident after fixation in modified Erliki's-formalin mixture. Guilliermond (1908) reported the retention, in the earlier stages of germination, of some of the aleurone grain protein which

[2] No attempt was made to differentiate plastid primordia from non-plastid-forming mitochondria before germination had begun.

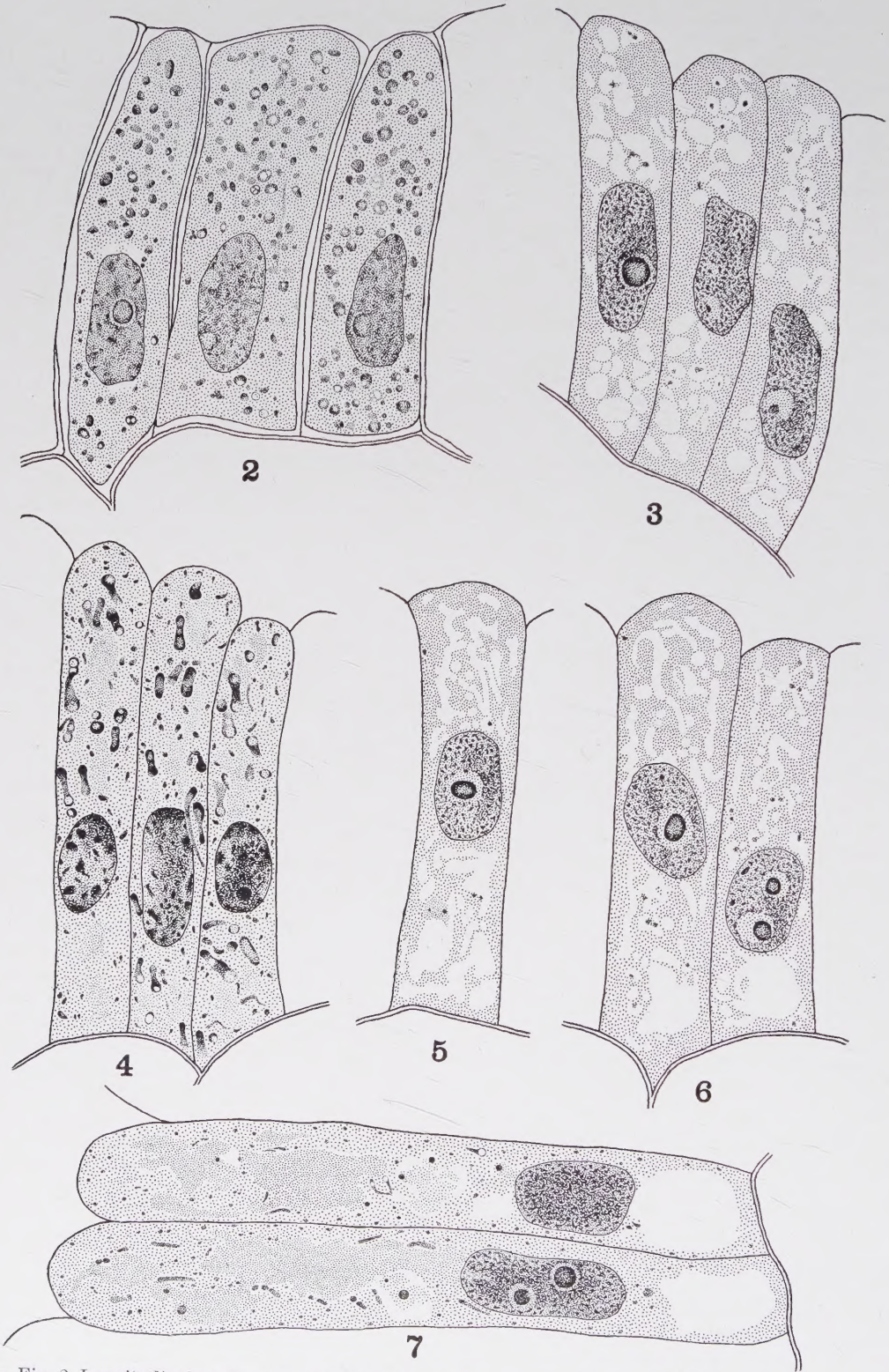

Fig. 2–7.—Fig. 2. Longitudinal section of epithelial cells in resting grain of *Triticum* fixed in modified Erliki's-formalin showing numerous small aleurone grains dispersed in the cytoplasm.—Fig. 3. Epithelial cells in germinating grain of *Triticum*. The radicle is just beginning to break through the outer grain covering. Modified Erliki's-formalin. Numerous small vacuoles formed from the dissolution of aleurone grains are evident. Some vacuoles still contain remnants of undissolved aleurone grain protein.—Fig. 4. *Secale*. Shoot 5 mm. Modified Erliki's-pyridine. Numerous large,

fused as the vacuoles coalesced. That these granules are of aleurone grain origin is doubtful, since they persist throughout the germination process and, at times, appear somewhat enlarged (fig. 10, 23, 25, 27, 31).

In a few specimens, fixation, particularly in modified Erliki's-formalin mixture, renders the cytoplasm and vacuolar material so homogeneous that the cell appears non-vacuolate except for the empty basal vacuole if it is present. That the distal region is vacuolate, however, can be concluded, since there is often present the above described, deeply staining, vacuolar granule. In such cases, the granule appears to be embedded in undifferentiated cytoplasm. Since so many other specimens show it to be contained in the vacuole, it can be concluded that the granule in this case is embedded in homogeneous vacuolar material which cannot be distinguished from the surrounding cytoplasm.

The mitochondrial and plastid elements dispersed in the cytoplasm retain the same general characteristics they displayed in stages I and II. In addition to the forms of plastids previously described, there are present occasionally small clusters of non-staining spheres held together in a thin, deeply staining matrix (fig. 11). The non-staining spheres are evidently starch grains.

Mitochondrial grouping.—In a number of cells at this stage, there is discerned, usually distad to the nucleus, a peculiar solitary grouping of large mitochondrial bodies. The attenuated elements extend in all directions from the center of a lightly staining, homogeneous mass and terminate in swollen head portions (fig. 11). The chance grouping of freely dispersed plastid primordia often renders identification of this structure difficult during this stage. However, in a number of cells, it is differentiated sufficiently from the dispersed plastid primordia to make identification definite. The difficulty in differentiating the structure from the plastid primordia occurring free in the cytoplasm may explain the failure to observe it in earlier stages. The grouping, to be more fully described, is better delineated in the following stage.

In many specimens, the cells have lengthened considerably during this stage and lateral separation, particularly of those cells in the anterior region of the layer, is evident.

Stage IV.—*Vacuoles.*—As the shoot increases in length from 15 to 30 mm., the various cytoplasmic inclusions become more distinct. Increased vacuolization characterizes the majority of cells. If the nucleus is basal, a vacuole extends from the nucleus to the distal end of the cell; if central, vacuoles are present in both the basal and distal regions (fig. 10, 16). The homogeneous vacuolar precipitate, described in stage III, is present in the cells of practically all specimens as a lightly staining mass which completely fills the vacuole (fig. 10, 16). This material is preserved by all the cytoplasmic fixatives used. While in any one specimen this precipitate characterizes the majority of cells, yet some cells contain practically empty vacuoles, housing at most a few irregular clumps of vacuolar material. As noted in stage III, many cells contain an empty basal vacuole, especially towards the end of this stage. In such cells, the homogeneous vacuolar precipitate often extends to the very limits of the clear vacuole. In many cases the granular cytoplasm is not visible between the two areas, so that they appear contiguous (fig. 16). In some cells the presence of a thin membrane is discernible (fig. 10, middle cell). At other times a definite zone of granular cytoplasm separates the two regions. Occasionally the homogeneous material extends throughout the entire length of the cell, even into the basal region (fig. 10). Frequently the homogeneous area is interrupted by the presence of a small, clear vacuole (fig. 10, 21) in which the spherical granule, noted in stage III, is often retained. Guilliermond (1908) reported the presence of a substance which stained in a diffuse manner in the vacuoles in the distal part of the cells from the fifth day until the end of germination. After an examination of his figures, it is to be concluded that the diffusely staining vacuolar material and the homogeneously precipitated material here reported are the same substance. As noted by Guilliermond (1908), this material persists until the end of germination, at which time the vacuoles appear empty. As indicated in stage III, a few specimens, notably those fixed in chromic sulfate-formalin, disclose the presence of only a small number of cells containing the homogeneous precipitate; in the majority of cells, the vacuoles are open, elongated cavities which stretch throughout the length of the cell and contain the precipitated vacuolar material in irregular clumps (fig. 12, 13). Other specimens fixed in the same killing fluids contain the homogeneous material in the majority of cells (fig. 10). The homogeneous material should be regarded as vacuolar and not as a non-granular portion of the cytoplasm, for the region occupied by this material is clearly vacuolar in earlier stages (fig. 3, 5, 6), and even in this stage it appears as a vacuole in certain cells, particularly after fixation in chromic sulfate-formalin. Some evidence to the contrary should be noted, however. In some instances, the material itself seems to contain vacuoles which appear empty when preserved by the usual cytoplasmic fixation (fig. 10, 21).

Mitochondria and plastids.—As in the previous stage, a profusion of the minute granular mitochondria is dispersed throughout the cytoplasm (fig. 14–

deeply staining plastid primordia are evident. The vesiculated forms are plastids. Small granular mitochondria are dispersed throughout the cytoplasm.—Fig. 5–6. *Secale.* Shoot 5 mm. Modified Erliki's-formalin. In the basal part of the cell, vacuoles are enlarging and coalescing. Note the presence of canalicular vacuoles in the distal part. Aleurone grain protein remnants are still evident.—Fig. 7. *Triticum.* Shoot 11 mm. Modified Erliki's-formalin. Enlarged vacuoles in the distal region of the cell are filled with a homogeneous precipitate. Large, empty basal vacuoles are evident. Note lightly staining plastid primordia and plastids. All figures ×1,550.

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19). Occasionally small filamentous mitochondria are present among the granular forms (fig. 17, 18). The plastid complex, in general, appears as previously described. The plastid primordia are usually present in the form of homogeneous rods (fig. 17, 19, 20, 21). The plastid assumes a variety of forms, frequently being preserved as a rod containing one or several small, optically clear vesicles either at the end of the rod or strung along its length (fig. 14–16, 18–19). Present in many cells are dense clusters of small vesicles embedded in a deeply staining matrix noted in stage III (fig. 15). In some cells, these bodies appear as a single, enlarged, spherical vesicle with a deeply staining cap on one side or a thin sheath stretched around its periphery (fig. 15). Occasionally the cap is elongated so that the whole structure has the appearance of a tennis racquet (fig. 14). The larger of these optically clear vesicles are readily demonstrated, by means of a polarizing microscope, to be starch grains. The smaller vesicles could not be so tested but it is highly probable that they are starch grains.

Mitochondrial grouping.—The previously mentioned (stage III) solitary grouping of mitochondria[3] is now readily discerned (fig. 17, 18, 20, 22, 24). The whole structure appears as a cluster of attenuated elements distinctly different in form from either the granular mitochondria or the plastid primordia present in the cells. Each element, of which there are a large but varying number in the cluster, is composed of a darkly staining, swollen head portion which tapers rapidly into an elongated, filamentous tail. The end of the tail is beyond the visibility of the microscope. The elements are usually so grouped that the tail portions arise in a lightly staining, optically homogeneous area (fig. 17, 18, 20, 24). The swollen head portions thus extend in all directions. Only rarely do any of the head portions appear vesiculated. Often the filamentous portions, while extending outward, are twisted and curved (fig. 17, 18). The elements retain the stain intensely after fixation in modified Erliki's-pyridine (fig. 17, 18, 20); it is on the basis of their preservation in this solution that they are here regarded as mitochondrial in nature. They stain only lightly after fixation in Bensley's mixture, as do the other cytoplasmic elements in the cell, and the head portions are not so swollen as they are after fixation in modified Erliki's-pyridine (fig. 24). In cells killed in modified Erliki's-formalin, the structure is only vaguely discernible, staining slightly more deeply than its cytoplasmic surroundings (fig. 22). It is not discernible after chromic sulfate-formalin fixation. The structure is not present in the granular cytoplasm; it is confined to a lightly staining homogeneous region the fixation image of which appears very similar to the previously described homogeneous material precipitated within the vacuole (fig. 17, 18, 24). This region appears, in many cases, to have been pulled away from the surrounding granular cytoplasm so that a thin,

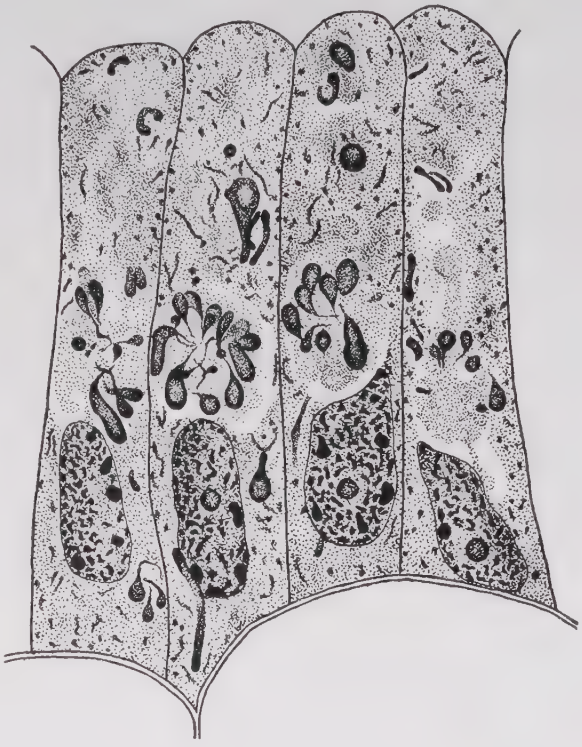
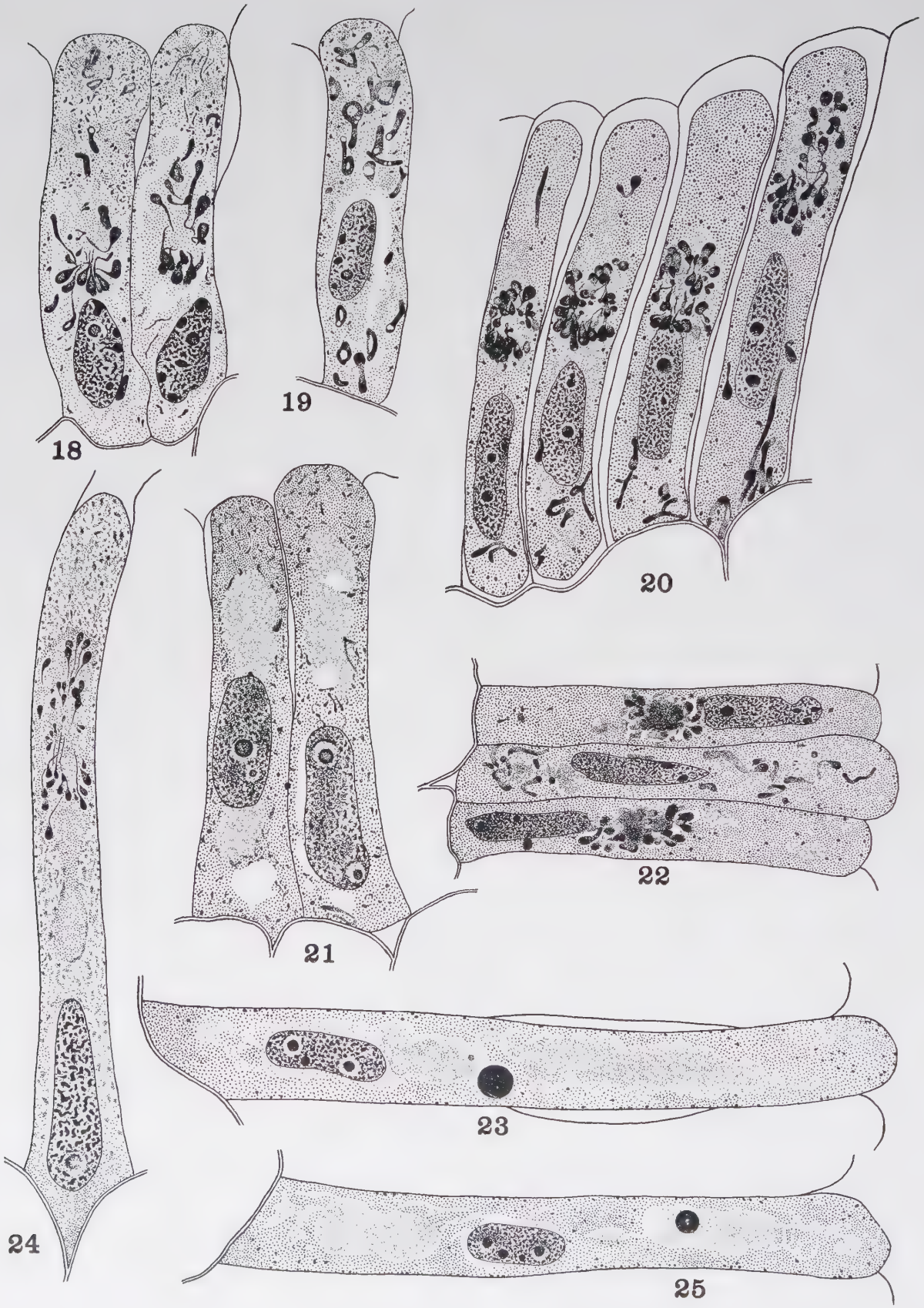


Fig. 17. *Secale.* Shoot 25 mm. Modified Erliki's-pyridine. Each cell contains the deeply staining, solitary mitochondrial grouping confined to a homogeneously precipitated area which appears vacuolar. Note plastid primordia, granular mitochondria and several small mitochondrial filaments. ×2,300.

[3] The labeling of these bodies as mitochondria is purely arbitrary. The term as here used merely indicates that they are preserved by those fluids which fix mitochondria and are dissolved by the fixatives which destroy mitochondria.

Fig. 8–16.—Fig. 8–9. *Triticum.* Shoot 11 mm. Chromium sulfate-formalin.—Fig. 8. Enlarged distal and basal vacuoles contain irregularly precipitated clumps of vacuolar material.—Fig. 9. Cell from the same section as cells in figure 8. The distal vacuoles contain the homogeneous precipitate. Note the presence of the empty basal vacuole.—Fig. 10. *Triticum.* Shoot 30 mm. Chromium sulfate-formalin. Large vacuoles, most of which are filled with the homogeneous precipitate, extend throughout the cells. Note the empty basal vacuoles. Small empty vacuoles are present in the homogeneous vacuolar precipitate in the cell on the right.—Fig. 11. *Triticum.* Shoot 12 mm. Modified Erliki's-pyridine. The mitochondrial grouping is evident in the left cell. The homogeneous area in which it is contained is not readily delineated. Note several plastids in addition to numerous plastid primordia and granular mitochondria.—Fig. 12–13. *Secale.* Shoot 29 mm. Chromium sulfate-formalin. Large extended vacuoles contain irregularly precipitated clumps of vacuolar material. Compare with figure 10. Note large vacuolar granules.—Fig. 14–15. *Secale.* Shoot 28 mm. Modified Erliki's-pyridine. Variously shaped plastids containing starch grains are present. Note granular mitochondria.—Fig. 16. *Triticum.* Shoot 20 mm. Bensley's. Large vacuoles containing the homogeneous precipitate and the empty basal vacuole are present. Note lightly staining plastid primordia and plastids. All figures ×1,550.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peripheral, clear zone is discerned after fixation in both modified Erliki's-pyridine and modified Erliki's-formalin (fig. 17, 18, 22). In some cells fixed in the latter solution the central portion of this region stains more deeply (fig. 22). When Bensley's fixative is used, this region is so fixed that it is in intimate contact with the granular cytoplasm. When thus fixed it is extremely difficult to delineate the region accurately (fig. 24). This is also true of the above mentioned precipitated vacuolar material (fig. 16.) Often the homogeneous region occupies little more area than the mitochondrial grouping contained within it (fig. 17). In other cases, the region is more expanded, extending beyond the grouping and appearing not unlike the previously described extended vacuole containing the homogeneous precipitate (fig. 24). It is difficult at times to determine whether or not the elements of the grouping extend beyond the homogeneous region into the surrounding cytoplasm. Usually, however, they appear to be confined within the region, although some of the head portions often extend to its very limits. In cells in which the grouping is more expanded, the greater number of elements extend necessarily toward the basal and distal ends of the cell because of the elongated cell shape (fig. 24). While in many cells the grouping is present just distad to the nucleus, its position varies from basal to distal. It appears only occasionally, however, in the region basal to the nucleus (fig. 22). The specimens varied in the number of cells in which the grouping could be demonstrated: in some, the greater number of cells contain the structure; in other, it could be delineated only occasionally. Since the fixation image of the homogeneous area housing the grouping appears very similar to the vacuolar homogeneous precipitate and since it is a definite zone set off from the granular cytoplasm, often being pulled away from it in fixation, it would appear that this region is vacuolar in nature. If this conclusion is correct, such a structure would indeed be unique in that it contains elements of mitochondrial nature which, moreover, assume a definite orientation, a condition in itself unique. It should be emphasized again, however, that future investigation may make it more convenient to consider this structure a highly specialized portion of the cytoplasm rather than a discrete vacuole.

As is evident from the accompanying figures, the cells of all specimens have not attained equal length at this period of germination. In some, the cells have reached a maximum length, and the lateral separation is pronounced; in others, the cells are apparently lengthening. There is a clear correlation between the length of the cells and their position in the epithelium: the cells are longer in the anterior region and shorter in the posterior. The gradation in length is regular. This difference in cell length persists until the end of germination. It is also in the anterior region that the cells attain greatest lateral separation, at the same time becoming highly irregular in shape, particularly in the final stages of germination. In some specimens this occurs to a lesser degree in the cells in the posterior region. In the majority of specimens, little or no separation or distortion characterizes the cells in the central region of the layer.

Stage V.—*Vacuoles.*—As germination continues, there is little change in the epithelial cells over an extended period, and it is not until the endosperm is almost depleted that more pronounced alterations occur. Thus, even when the shoots have reached a length of 7 or 8 cm., the condition of the cells in many specimens varies little from that in the last described stage. The usually empty, basal vacuole is present in the majority of cells, although even at this stage it is of infrequent occurrence in some specimens. Its presence was also reported by Guilliermond (1908) as characteristic of the later stages of germination. In some of these vacuoles an irregular precipitate occurs. The majority of cells still show the extended areas of lightly staining, homogeneous vacuolar precipitate, which, as previously noted, is often contiguous with the empty, basal vacuole or, when the latter is not present, frequently extends into the basal region of the cell (fig. 25). When the nucleus occurs in the distal region of the cell, the homogeneous area often extends from the proximal end of the nucleus to the empty basal vacuole. In a few cases, when the empty vacuole is not completely basal, the homogeneous area borders on both its distal and proximal sides. In some specimens the homogeneous material appears to have been pulled away from the vacuolar membrane, probably as the result of fixation (fig. 23, 25).

In a number of cells in the anterior region of the epithelial layer of many specimens, the vacuoles do not contain the characteristic homogeneous precipitate but instead contain irregular clumps of precipitated material. In a few specimens this condition appears in cells dispersed throughout the whole layer. In other grains, particularly toward the end of this stage, this condition is characteristic of the majority of cells (fig. 27), and only an occasional cell

Fig. 18–25.—Fig. 18–19. *Secale.* Shoot 25 mm. Modified Erliki's-pyridine. Cells from the same specimen as the cells illustrated in figure 17.—Fig. 18. Note the varying form of the mitochondrial grouping when compared with figure 17.—Fig. 19. The mitochondrial grouping is not present. Note the variously shaped plastids and the granular mitochondria.—Fig. 20. *Triticum.* Shoot 25 mm. Modified Erliki's-pyridine. The cytoplasmic inclusions are similar to those illustrated in figures 17–18, although the homogeneous area containing the mitochondrial grouping is not as sharply delineated.—Fig. 24. *Triticum.* Shoot 20 mm. Bensley's. The lightly staining mitochondrial grouping is evident. The cell is from the same specimen as the cell illustrated in figure 16.—Fig. 21. *Triticum.* Shoot 29 mm. Bensley's. Note the small empty vacuoles contained in the homogeneous vacuolar precipitate.—Fig. 22. *Secale.* Shoot 25 mm. Modified Erliki's-formalin. Two cells contain the mitochondrial grouping. Note the deeply staining center of the grouping. Lightly staining plastid primordia are present in the middle cell.—Fig. 23 and 25. *Secale.* Shoot 60 mm. Modified Erliki's-formalin. Extending throughout the cell are large vacuoles containing the homogeneous precipitate. Note the large, deeply staining vacuolar granule embedded in the homogeneous material. All figures ×1,550.

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displays the homogeneous precipitate. Since the vacuoles are usually empty at the end of germination this condition must indicate a gradual depletion of the vacuolar material that is precipitated in fixation.

There is no marked change in either the plastid or mitochondrial complex at this stage. In many cells the plastids contain enlarged starch grains; in others the typical plastid primordia are retained. The number of granular mitochondria has not decreased when this stage of germination is reached. There is little change in the peculiar mitochondrial grouping, either in form or position (fig. 26).

Stage VI.—*Vacuoles.*—At the end of germination, as the endosperm becomes emptied, the shoots attain a length of from 12 to 15 cm. At this stage the cells are highly vacuolate, the vacuoles appearing empty or at most containing a few irregular clumps of precipitated material (fig. 31). In many cells only a thin peripheral layer of cytoplasm is present so that large, empty vacuoles occur on either side of the nucleus, a condition also observed by Guilliermond (1908). In cells which are evidently beginning to disintegrate, the nucleus stains irregularly and in some specimens becomes rounded, while in others it assumes an irregular form (fig. 29, 30). In other specimens the contents of some of the cells appear to have dissolved so that little is left except the cell wall.

Plastids and mitochondria.—The plastid and mitochondrial elements evidently persist until the cells begin to disintegrate. A number of the large plastid primordia are still present in many cells (fig. 29, 30). A definite plastid with contained starch grains is discernible in rare instances. The granular mitochondria are still evident (fig. 29, 30). Horning and Petrie (1927) reported the disappearance of the migratory mitochondria as germination draws to a close, a condition they identified indirectly with cessation of secretory activity. It is to be noted, however, that on the completion of the germination process the epithelial cells begin to disintegrate, and it is quite possible that to this condition is due the disappearance of cytoplasmic inclusions.

Mitochondrial grouping. — The mitochondrial grouping can still be demonstrated in some cells at this stage. It is now greatly contracted, appearing as a darkly staining rosette. From the usually deeply staining, undifferentiated center, the head portions project in all directions (fig. 29, 30). The elongated tails have evidently contracted to draw the head portions together. The homogeneous area in which the grouping is contained in earlier stages is here not discernible. While the exact position of the dense grouping in relation to the vacuole is at times difficult to discern, there is evidence to indicate that it is present within the vacuole. Usually it appears to be confined within an area devoid of cytoplasm which evidently is a vacuole. While a portion of the rosette may extend to the cytoplasm, the greater part appears free in the vacuole (fig. 29, 30). It is to be noted that in a specimen of comparable shoot length in which the epithelial cells are not so advanced as here described, the grouping in most cells appears as in earlier stages and is contained within the homogeneous area. In a few cells, however, the grouping, although still within the homogeneous area, assumes the contracted form characteristic of the final stage (fig. 28). In still other cells, while the greater number of elements are contracted to form a dense rosette, a few retain the elongated form and project a distance beyond the main grouping, but are still contained within the homogeneous area (fig. 32).

With endosperm depletion, the secreting and absorbing activity of the epithelial cells comes to an end. This cessation of function is accompanied by an obvious disintegration of the specialized structure which characterizes the cells. The cells themselves soon die and disintegrate.

Discussion.—While it is evident that the epithelial cells of the scutellum are gland cells, the changes occurring during activity and the presence of specific structures must be interpreted in terms of two functions, since the cells absorb the digested endosperm material as well as secrete enzymes.

Vacuoles.—Neither the structure nor the position of the vacuoles in the epithelial cells give positive evidence that they play a rôle either in secretion or absorption. The nature of the precipitated homogeneous material present within the vacuoles is as yet undetermined. It is highly probable that some of the soluble carbohydrates absorbed from the endosperm collect in the vacuoles, but such a substance would not be precipitated by the type of fixation here used. The fact that the material is precipitated by the mitochondrial fixatives indicates perhaps that it is of a protein nature. Such a supposition suggests several possibilities as to its origin. That it might be a fixation image of a fine dispersion of a colloidally dispersed protein which remains within the vacuoles after the dissolution of the aleurone grains is extremely doubtful. Such a conclusion, moreover, would not be supported by the earlier observations of Guilliermond (1908). Another possibility is that the precipitated substances is actually an enzyme, pos-

Fig. 26–32.—Fig. 26. *Triticum.* Shoot 95 mm. Bensley's. The mitochondrial grouping contained within the homogeneously precipitated area is present.—Fig. 27. *Triticum.* Shoot 80 mm. Modified Erliki's-formalin. Large vacuoles extending throughout the cell contain irregularly precipitated clumps of vacuolar material. Note the vacuolar granules.—Fig. 28 and 32. *Triticum.* Shoot 110 mm. Modified Erliki's-pyridine. A solitary contracted mitochondrial grouping is contained within the homogeneous area. In figure 32 some of the elements of the contracted grouping are still extended, their form appearing very similar to that of earlier stages.—Fig. 29–30. *Triticum.* Shoot 150 mm. Modified Erliki's-pyridine. In two cells the mitochondrial grouping is greatly contracted and now no longer appears to be contained within the homogeneous area. The vacuoles are empty. Note plastid primordia and granular mitochondria.—Fig. 31. *Triticum.* Shoot 150 mm. Modified Erliki's-formalin. The greatly extended vacuoles contain only a few irregularly precipitated clumps of vacuolar material. Note vacuolar granules. All figures ×1,550.

sibly a protein, produced by the epithelial cells and collected in the vacuoles before it is discharged, in some unknown manner, into the endosperm. As previously noted, this homogeneous material is concentrated particularly in the distal region of the cell and, as the endosperm approaches complete depletion, it tends to disappear.

The position of the empty, basal vacuole suggests that it functions in absorption. The soluble carbohydrate material absorbed from the endosperm by the epithelial cell may collect temporarily in this vacuole from which it is eventually carried away through the scutellar parenchyma to the growing plant.

Plastids and mitochondria.—Of the three types of inclusions preserved by the mitochondrial fixatives in the epithelial cells during germination, a definite function can be assigned to only one. The large, usually rod-shaped bodies are plastid primordia. These inclusions readily develop into plastids as optically clear vesicles which can be identified as starch grains form within them. In typical meristematic cells, two categories of mitochondria have been recognized, the one composed of plastid primordia, the other including the forms not developing into plastids. Satisfactory criteria for differentiating the two categories are still unavailable. In the epithelial cells, however, plastid primordia can be differentiated on the basis of size and shape from non-plastid-forming mitochondria. It is concluded that all of the large, usually rod-shaped inclusions are plastid primordia, since in addition to the fact that it is only from this type of inclusion that the plastids arise, there is the further evidence presented in some cells in which practically all of these bodies have developed into plastids (fig. 14, 19). The presence of such structures can be interpreted in terms of absorption only, for they function as a temporary storage place for starch which is later reabsorbed as germination draws to a close.

No definite function can be assigned to the numerous minute granular mitochondria present throughout germination. It is possible that they belong in the same category as the non-plastid-forming types of mitochondria found in meristematic cells and have arisen from such types as the development of the epithelial cells proceeds from undifferentiated meristematic tissue. The evidence at hand, other than their existence in large numbers, does not warrant an assumption that they here acquire a function connected with the specialized activity of the epithelial cells.

Mitochondrial grouping.—The grouping of the large mitochondrial elements in the epithelial cells is unique. The elements themselves are peculiar in that, when fixed, they always assume a form in which a definite head and tail can be differentiated. In addition, the elements are definitely oriented in a characteristic group. They are not present in the granular cytoplasm but are contained within a homogeneous region which the evidence at hand indicates to be a highly specialized type of vacuole. The structure as here described has been observed only in fixed cells, so what has actually been seen is a fixation image of a cell inclusion that may have a somewhat different form in the living cell. However, since several types of fixative preserve the structure with little change in form, its fixation image may approximate very closely its structure in the living cell. As far as is known, such a structure has not been previously reported as occurring in any other type of cell. The evidence at present does not warrant the assigning of a particular function to this structure; but the fact that it occurs only in specialized glandular cells perhaps indicates that it is associated in some way with secretory activity. Such an assumption must be made with caution, however, for in the past, the mere presence of a particular body within a cell has led many investigators to assign a specific function to the body without the support of critical evidence.

It must be noted that there is some indication of the presence of the mitochondrial grouping in the sub-epithelial layers of the parenchyma cells of the scutellum as well as the other types of inclusion and the homogeneous vacuolar material. A cytological investigation of these cells is in progress.

SUMMARY

The epithelial cells of the scutellum of *Triticum sativum* and *Secale cereale* are organs of secretion and absorption. The internal changes occurring in the cells during the course of their activity and the presence of specific structures are considered in terms of these two functions.

As germination begins, small vacuoles form in the cells through the swelling and dissolution of aleurone grains present in the resting stage. As germination progresses, these vacuoles enlarge and coalesce. In the large vacuoles thus formed, there appears a vacuolar material which is precipitated in fixation as a lightly staining homogeneous mass. This material, possibly protein in nature, disappears at the end of germination, at which time the cells are completely vacuolate and devoid of vacuolar material.

Plastid primordia, separated on the basis of size and shape from non-plastid-forming mitochondria, are present in the epithelial cells during germination. These bodies readily develop into plastids as starch grains form within them.

Present also in the epithelial cells are two types of non-plastid-forming mitochondria. The first is represented by a profusion of minute granular bodies of unknown function occurring free in the cytoplasm. The second type of mitochondria, so designated because it is preserved by certain fixatives, does not occur freely dispersed; instead, these elements, each composed of a filament and enlarged terminal portion, are localized in a characteristic solitary group. The filamentous portions project in all directions from the center of the group and terminate in swollen heads. The structure is not present in the granular cytoplasm but is contained within a homogeneous region indistinguishable from the precipitated homogeneous vacuolar material. While this region may be a highly specialized portion of the cytoplasm, the

evidence at hand indicates that it is vacuolar in nature. Relationship between the structure and secretion is suggested.

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